

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:)	
Alan L. Mueller, et al.)	
Serial No.: 09/990,405)	Examiner: Kwon, Brian Yong S.
Filing Date: November 21, 2001)	Group Art Unit No.: 1614
For: METHODS AND COMPOUNDS FOR)	
TREATING DEPRESSION AND)	
OTHER DISORDERS)	

DECLARATION UNDER RULE 132 OF ALAN L. MUELLER

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Alan L. Mueller hereby declares:

1. I am the principal inventor named in the above-referenced patent application.
2. I am currently employed as Vice President of Research at NPS Pharmaceuticals, Inc., and serve as Adjunct Associate Professor in the Department of Pharmacology and Toxicology at the University of Utah School of Medicine. I have a Bachelor of Science degree in Pharmacy from the University of Kansas, and a Ph.D. in Pharmacology from the University of Colorado Health Sciences Center. A copy of my curriculum vitae, providing additional details of my professional and technical experience and expertise, is attached as Exhibit A.
3. I have reviewed the Office Action mailed August 10, 2006, in the present application, and the references cited by the examiner, namely, Mueller et al. (WO 96/40097) and Skolnick et al. (Pharmacopsychiatry, Abstract, 1996 January, 29:1, 23-6).
4. It is my understanding that the Office Action asserts that the invention claimed in this application is unpatentable over Mueller et al. (WO 96/40097) and Skolnick et al. (Pharmacopsychiatry, Abstract, 1996 January, 29:1, 23-6), because the claimed methods for treating depression using the recited compounds would have been obvious to one of ordinary skill in the art. Specifically, the Office Action states that:

- (i) the Mueller et al. reference teaches that some compounds recited in the present application's method of use claims are NMDA receptor antagonists,
- (ii) the Skolnick et al. reference teaches that certain compounds having clinically effective antidepressant activity are all active at the NMDA receptor, and concludes that,
- (iii) therefore, one skilled in the art would have had a reasonable expectation that the compounds used in the methods of the present invention (having NMDA antagonist activity) would also be expected to have antidepressant activity.

It is my understanding, based on the above line of reasoning, that it is the position of the Examiner that if *some* compounds having antidepressant activity are active at the NMDA receptor, then *all* compounds active at the NMDA receptor would be expected to have antidepressant activity. This position is unfounded and incorrect, and one skilled in the art, like myself, would not draw these conclusions for the reasons I explain below.

5. First, from a scientific point of view, the mere fact that *some* compounds having antidepressant activity are active at the NMDA receptor does not mean that *all* compounds active at the NMDA receptor would be expected to have antidepressant activity. While this data, from a limited group of antidepressants, may lead one skilled in the art to postulate or hypothesize a general rule that all NMDA antagonists are antidepressants, the data is not sufficient to establish a reasonable scientific basis for concluding that all NMDA antagonists are antidepressants. In fact, even the Skolnick reference itself acknowledges that the data is, at best, hypothetical. Specifically, Skolnick states, hypothetically, that "*we propose that adaptive changes in NMDA receptors may be the final common pathway for antidepressant action.*" (Abstract; emphasis added). Clearly, the mere fact that 17 different compounds known to have antidepressant activity were active at the NMDA receptor, however, does not imply that *all* compounds known to have antidepressant activity are active at the NMDA receptor or that *all* compounds active at the NMDA receptor will also have antidepressant activity. Due to the complexity of biological systems, it is possible that some compounds active at the NMDA receptor may not have antidepressant activity.

6. Second, empirical data in the art and data obtained from my own research indicates that some compounds active at the NMDA receptor do *not*, in fact, have antidepressant activity. I submit with this Declaration Exhibits B, C, and D, which provide evidence that some compounds active at the NMDA receptor do not, in fact, have antidepressant activity.

7. Exhibit B shows experimental data generated by NPS Pharmaceuticals, under my supervision, indicating that six compounds 50, 65, 118, 119, 156, and 186 in the present application, are active at the NMDA receptor, but do *not* possess statistically significant antidepressant activity. The compounds shown in Exhibit B were assayed in a forced-swim test (FST), substantially as described in Example 2 of the Specification of the present application (Exhibit B, last column 9). The same compounds were also assayed in a [³H]MK-801 NMDA receptor binding assay, substantially as described in Example 1 of the Specification of the present application (Exhibit B, column 4). The data in Exhibit B shows that compounds 50, 65, 118, 119, 156, and 186 in the application bound to the NMDA receptor (IC₅₀ (μM) vs. NMDA [³H]MK-801 values of 0.762, 2.0, 0.240, 0.087, 0.090, and 0.123, respectively), indicative of NMDA receptor binding. The data in Exhibit B further shows that compounds 50, 65, 118, 119, 156, and 186 in the application did not show statistically significant antidepressant activity in the mouse FST assay (showing a 38%, 33%, 21%, 32%, 14%, and 33% decrease, respectively, in the duration of immobility in the forced-swim test following administration of the test compounds). Based on the results of standard statistical analysis as well as my own experience, a 38% or less decrease in the duration of immobility in the forced-swim test following administration of the test compounds is not statistically or clinically predictive of antidepressant activity. In conclusion, the above data shown in Exhibit B demonstrates that six compounds, compounds 50, 65, 118, 119, 156, and 186 in the application, are active at the NMDA receptor but do *not* possess statistically or clinically significant antidepressant activity. This contradicts that Examiner's assertion that NMDA receptor binding activity is predictive of antidepressant activity.

8. In addition, Exhibit C is a scientific publication by Panconi et al. (*Pharmacology Biochemistry and Behavior*, Vol. 46, pp. 15-20, 1993), which also shows experimental data indicating that certain NMDA receptor antagonists do not possess antidepressant activity. Specifically, Panconi et al. teach that "investigations ([in the] reserpine, apomorphine, and yohimbine tests) could not confirm the suspected antidepressant activity" and that "other NMDA antagonists – 2-amino-7-phosphonoheptanoic acid (AP7), kynurenic acid, and 1-glutamic acid diethylester (GDEE) – showed no activity in the TST" (a tail suspension test of antidepressant activity), and conclude that "these findings throw doubt concerning the potential antidepressant activity of MK-801 and other NMDA antagonists" (Abstract). Panconi et al. further state that a

"definitive answer as to whether functional antagonists at the NMDA receptor complex represent potential antidepressants cannot be given" (page 19, last paragraph). Thus, Panconi et al. provide additional examples of NMDA receptor antagonist compounds that do not have antidepressant activity. Based on the above teachings of Panconi et al., I conclude that one of ordinary skill in the art would not have had a reasonable expectation that NMDA receptor antagonist activity is predictive of antidepressant activity.

9. In further support of the proposition that NMDA receptor antagonist activity is not predictive of antidepressant activity, I refer to Exhibit D, a scientific publication by Zarate et al. (*Am. J. Psychiatry* 163:153-155, January 2006), which discloses a parallel-group, placebo-controlled trial of a selective NMDA receptor antagonist, memantine, to determine efficacy in the treatment of major depressive disorders. Zarate et al. state that "low-to-moderate-affinity NMDA antagonist memantine in doses of 5-20 mg/day was not effective in the treatment of major depressive disorder" (Abstract) and that "although it is possible that higher doses of memantine may have resulted in significant antidepressant effects, the dose chosen for the present study was judged to be sufficient to test the validity of the concept of NMDA receptor antagonism with memantine" (Discussion section). Thus, Zarate et al. provide additional examples of NMDA receptor antagonist compounds that do not have antidepressant activity.

10. In summary, Exhibits B, C, and D each provide evidence that certain NMDA receptor antagonist compounds do not have antidepressant activity. These teachings contradict the assertion of the Examiner that one of ordinary skill in the art would have expected that NMDA receptor antagonists would have antidepressant activity. The scientific data available in the published literature, together with the data generated at NPS, taken as a whole, shows that some NMDA receptor antagonists have antidepressant activity, while other NMDA receptor antagonists do not have antidepressant activity. The scientific data does not, therefore, support the conclusion that all NMDA receptor antagonists would be expected to have antidepressant activity. Based on my review of the references cited by the Examiner, together with the data shown in Exhibits B, C, and D, it is my conclusion that one of ordinary skill in the art would not have had a reasonable expectation that compounds having NMDA receptor antagonist activity would be expected to have antidepressant activity.

11. All statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true. These statements were made with the

knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Signed: Alan L. Mueller
Alan L. Mueller

Date: 12 Feb 2007

EXHIBIT A

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CURRICULUM VITAE
ALAN L. MUELLER

Home Address

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Birthplace: Kansas

Birthdate: June 28, 1954

Business Address

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Education

May, 1977:

B.S. (Pharmacy)
University of Kansas
Lawrence, Kansas

March, 1982:

Ph.D. (Pharmacology)
University of Colorado Health Sciences Center
Denver, Colorado

Positions Held

June, 1977 - March, 1982:

Predoctoral Trainee
Department of Pharmacology
University of Colorado Health Sciences Center
Denver, Colorado

May, 1982 - April, 1984:

Postdoctoral Fellow
Department of Neurological Surgery
University of Washington
Seattle, Washington

May, 1984 - October, 1984:

Research Associate
Department of Neurological Surgery
University of Washington
Seattle, Washington

November, 1984 - January, 1989:

Pharmacologist
Neuroscience Research Division
Pharmaceutical Discovery
Abbott Laboratories
Abbott Park, Illinois

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February, 1989 - February, 1992: Research Scientist
NPS Pharmaceuticals, Inc.
Salt Lake City, Utah 84108

March, 1992 – September, 1999: Scientist IV and Project Team Leader
NPS Pharmaceuticals, Inc.
Salt Lake City, Utah 84108

September, 1999 – December, 2000: Director, Drug Discovery
NPS Pharmaceuticals, Inc.
Salt Lake City, Utah 84108

January, 2001 – September, 2005: Vice President, Drug Discovery
NPS Pharmaceuticals, Inc.
Salt Lake City, Utah 84108

September, 2005 – present: Vice President, Research
NPS Pharmaceuticals, Inc.
Salt Lake City, Utah 84108

July, 1996 - present: Adjunct Associate Professor
Department of Pharmacology and Toxicology
University of Utah School of Medicine
Salt Lake City, UT 84112

Professional Societies and Organizations

American Academy of Neurology (Affiliate Member)
American Association for Laboratory Animal Science (AALAS)
American Heart Association (Stroke Council)
American Pain Society
National Stroke Association
Neurotrauma Society
Society for Neuroscience

Academic Awards and Honors

Veta B. Lear Award	1973
Undergraduate Research Award	1976
Pharmacology Student Award	1977
Pharmacy Gold Key Award	1977

Grant Support

NIH Postdoctoral Grant Awardee	1982
NIH Phase I SBIR Grant Awardee	1993
NIH Phase I SBIR Grant Awardee	1995

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31. Li, H., R.L. Balster and A.L. Mueller. Lack of PCP-like effects of argitoxin-636, a polyamine toxin NMDA receptor antagonist. *1996 College on Problems of Drug Dependence*, Virginia Commonwealth University, summer, 1996.
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Calcium Channels Useful for Treatment of Neurological Disorders and Diseases. US
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Available upon request

EXHIBIT B

Compound	Structure	IC ₅₀ (μM) vs. NMDA (RCGC)	IC ₅₀ (μM) vs. NMDA (³ H]MK-801)	5-HT reuptake K _i =	NE reuptake K _i =	DA reuptake K _i =	Central Muscarinic, nonselective K _i =	Activity in Mouse FST (p.o.)
NPS 846 Application Cpd 20		0.070	0.252	K _i = 256 nM	K _i = 312 nM	K _i = 3.0 μM	K _i = 348 nM	60% decrease at 30 mg/kg
NPS 1407 Application Cpd 50		0.089	0.762	K _i = 45 nM	K _i = 107 nM	K _i = 1.8 μM	K _i = 1.6 μM	38% decrease at 30 mg/kg
NPS 1512 Application Cpd 65		0.167	2.0	K _i = 127 nM	K _i = 306 nM	K _i = 22 μM	K _i > 10 μM	33% decrease at 30 mg/kg
NPS 1819 Application Cpd 118		0.409	0.240	K _i = 131 nM	K _i = 262 nM	K _i = 4.1 μM	K _i = 882 nM	21% decrease at 30 mg/kg
NPS 1820 Application Cpd 119		0.115	0.087	K _i = 30 nM	K _i = 416 nM	K _i = 1.6 μM	K _i = 2.5 μM	32% decrease at 30 mg/kg

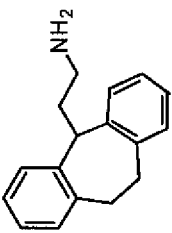
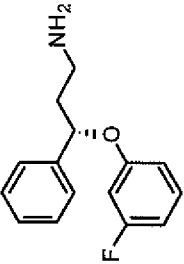
Compound	Structure	IC ₅₀ (μM) vs. NMDA (RCGC)	IC ₅₀ (μM) vs. NMDA ([³ H]MK-801)	5-HT reuptake	NE reuptake	DA reuptake	Central Muscarinic, nonselective	Activity in Mouse FST (p.o.)
NPS 1913 Application Cpd 156		0.069	0.090	K _i = 1.2 μM	K _i = 570 nM	K _i > 10 μM	K _i = 1.7 μM	14% decrease at 30 mg/kg
NPS 2115 Application Cpd 186		0.155	0.123	K _i = 22 nM	K _i = 136 nM	K _i = 3.1 μM	K _i = 405 nM	33% decrease at 30 mg/kg
memantine		~500 nM						64% decrease at 30 mg/kg

EXHIBIT C

MK-801 and Enantiomers: Potential Antidepressants or False Positives in Classical Screening Models?

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PANCONI, E., J. ROUX, M. ALTENBAUMER, S. HAMPE AND R. D. PORSOLT. *MK-801 and enantiomers: Potential antidepressants or false positives in classical screening models?* PHARMACOL BIOCHEM BEHAV 46(1) 15–20, 1993.—In the present experiments, the noncompetitive NMDA antagonist 5-methyl-10,11-dihydroxy-5H-dibenzo(a,d)cyclo-hepten-5,10-imine (MK-801) and its (+) and (–) enantiomers were tested in classical screening models used to detect potential antidepressants. The drug and its enantiomers were active in the tail suspension test (TST). The racemate was also active in the forced swimming test (FST). The effects in these tests occurred, however, at doses with marked stimulant activity. Further investigations (reserpine, apomorphine, and yohimbine tests) could not confirm the suspected antidepressant activity. Other NMDA antagonists—2-amino-7-phosphonoheptanoic acid (AP7), kynurenic acid, and L-glutamic acid diethylester (GDEE)—showed no activity in the TST. These findings throw doubt concerning the potential antidepressant activity of MK-801 and other NMDA antagonists.

NMDA antagonists	Antidepressants	Screening models	Mice	MK-801	Enantiomeric forms
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THE vast majority of synapses in the CNS appear to use excitatory amino acids (EAAs) such as glutamate and aspartate as their neurotransmitters (15). Recent attention has focused primarily on the NMDA receptor, mainly found in the CA1 cell body layer of hippocampus (23). NMDA receptor activity appears to underlie a number of complex neurophysiological phenomena and administration of antagonists at the NMDA receptor complex can produce anxiolytic and anticonvulsant effects in various animal models (5–7,9).

Of particular interest are the reported effects of NMDA antagonists in models related to depression. Trullas and Skolnick (29) reported that the NMDA antagonists 5-methyl-10,11-dihydroxy-5H-dibenzo(a,d)cyclo-hepten-5,10-imine (MK-801) and 2-amino-7-phosphonoheptanoic acid (AP7) decrease the immobility induced in mice in either the forced swim test (FST) (18) or tail suspension test (TST) (24,25). As both models expose animals to inescapable aversive situations, a related finding is that NMDA antagonists also antagonize stress-induced increases in dopamine (DA) metabolism (22). Other evidence has shown that NMDA receptor activity and stress-induced behavioral change are involved in long-term potentiation (LTP). LTP, an increase in synaptic efficacy, occurs when the NMDA receptor is activated (8,10) and has

also been associated with exposure to inescapable stress (23). It has thus been suggested that specific pathways subserved by NMDA receptors may have a modulating role in affective disorders and that NMDA antagonists may represent a novel class of antidepressant (29).

The present experiments were undertaken therefore to enlarge on previous studies using MK-801 racemate and its enantiomers. In addition to the FST and the TST, MK-801 and its enantiomers were studied in an activity meter test. MK-801 racemate was also tested for potential antidepressant activity in pharmacological tests such as reserpine antagonism, high-dose apomorphine antagonism, and potentiation of yohimbine lethality. Further studies investigated the effects of other well-known NMDA antagonists—AP7, kynurenic acid, and L-glutamic acid-diethylester (GDEE)—in the TST.

METHOD

Animals

Male OF1 mice (20–35 g) (IFFA-CREDO l'Arbresle, France) were housed in 15.5 × 31 × 14-cm plastic cages (six per cage) with food and water freely available for at least 1 week before testing. Animals were moved from the housing

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colony room to the testing area 12 h before testing to adapt to the new environment. In cases of oral drug administration, they were deprived of food 12 h before testing. The animal house and laboratories were maintained at a temperature of $22 \pm 1^\circ\text{C}$ and a relative humidity of $65 \pm 15\%$ with a noninverted 12 L : 12 D cycle (light on at 7:00 a.m.).

FST

The FST was performed following the procedure described by Porsolt et al. (18). The drug was administered SC and 30 min later mice were placed individually in a transparent vessel (height: 25 cm, diameter: 10 cm) filled with 6 cm water ($23\text{--}25^\circ\text{C}$). After 2 min of adaptation, the duration of immobility was measured over a period of 4 min. Mice were considered immobile when they made no further attempts to escape except the movements necessary to keep their heads above water. Each experimental group consisted of 10 animals. Control animals received only the vehicle.

TST

The TST was performed using an automated device (ITEMATIC-TST) described by Stéru et al. (25). The drugs were administered SC or PO 30 min before the 6-min test. For testing, mice were suspended by the tail using adhesive tape (20 mm from the tail's extremity). The total duration of immobility was recorded for the duration of the test. Each experimental group consisted of 10 animals. Control animals received only the vehicle.

Locomotor Activity

Locomotor activity was investigated to evaluate whether the drugs used induce sedation or locomotor stimulation following the procedure described by Boissier and Simon (2). Immediately after drug administration, mice were placed in a standardized transparent box, fitted with criss-cross photoelectric beam circuits. Locomotor activity was automatically measured by counting the interruptions of the photoelectric beams over a 30-min period. Each experimental group consisted of 12 animals. Control animals received only the vehicle.

Antagonism of Reserpine-induced Hypothermia

Antagonism of reserpine-induced hypothermia was studied following the procedure described by Bourin et al. (4). Drugs were administered PO 4 h after an IP injection of reserpine (2.5 mg/kg). Rectal temperature was measured, using an electrothermal probe inserted 1.5 cm into the rectum, immediately before drug administration and 60 min and 120 min after drug administration. Each experimental group consisted of six animals. Control animals received only the vehicle after reserpine.

Antagonism of High-dose Apomorphine

Antagonism of high-dose apomorphine-induced hypothermia was studied following the procedure described by Puech et al. (20). The compounds were administered PO 30 min before apomorphine. Rectal temperature was measured, as described above, 30 min before and 30 min after apomorphine administration. Each experimental group consisted of six animals. Control animals received only the vehicle before apomorphine.

Potentiation of Yohimbine Lethality

Potentiation of yohimbine lethality was studied according to the procedure described by Quinton (21). Drugs were administered 30 min before yohimbine (25 mg/kg, SC). The percentage of lethality of each group was recorded 24 h after treatment. Each experimental group consisted of 10 animals. Control animals received only the vehicle before yohimbine.

Statistical Procedures

Data were analyzed statistically by comparing drug-treated groups with control using the Dunnett test.

Drug Administration

Drugs and vehicle were administered SC or PO in a constant volume of 10 ml/kg body weight. Drugs were administered PO except for the experiments with MK-801 in the FST and AP7 in the TST, where they were administered SC. The SC route was chosen for the latter two experiments to be closer to the conditions used by Trullas and Skolnick (29), where these drugs were injected IP. In all cases doses are expressed as base. The following compounds were used: MK-801 (Research Department, Laboratoires Sarget, Mérignac, France), (–)MK-801 hydrogen maleate (RBI Bioblock, Research Biochemicals, Inc., Natick, MA), (+)MK-801 hydrogen maleate (RBI Bioblock), GDEE HCl (Sigma Chemical Co., St Louis, MO), AP7 (RBI Bioblock), kynurenic acid (RBI Bioblock), yohimbine (Sigma), apomorphine (Sigma), and reserpine (Sigma). All drugs were suspended in Tween 5% (Tween-80, Prolabo, Paris, France) and diluted with saline (SC) or distilled water (PO). Reserpine was dissolved in three to four drops of glacial acetic acid (Farmitalia Carlo-Erba, Milan, Italy) before dilution with distilled water. Apomorphine was dissolved in distilled water.

RESULTS

The effects of (±)-MK-801 in the TST are shown in Fig. 1. A dose-dependent decrease in immobility was observed in the dose range 0.1–1 mg/kg PO.

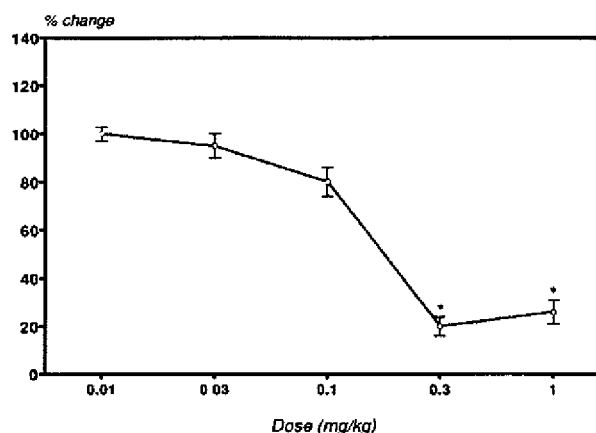


FIG. 1. Effects of (±)-5-methyl-10,11-dihydroxy-5H-dibenzo(a,d)cyclohepten-5,10-imine (MK-801) (expressed as % change from control \pm SEM) on immobility time in the tail suspension test (TST). The control mean was 150.1 ± 14.4 . (±)MK-801 was administered PO 30 min before testing. *Significantly different from control group ($p < 0.05$, Dunnett test).

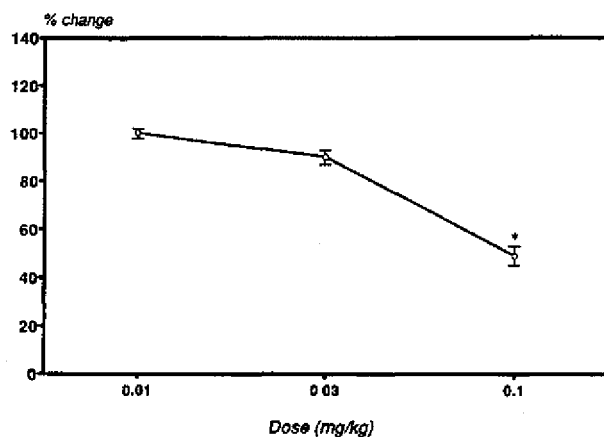


FIG. 2. Effects of (\pm)5-methyl-10,11-dihydroxy-5*H*-dibenzo(*a,d*)cyclohepten-5,10-imine (MK-801) (expressed as % change from control \pm SEM) on immobility time in the forced swimming test (FST). The control mean was 189.1 ± 18.0 . (\pm)MK-801 was administered SC 30 min before testing. *Significantly different from control group ($p < 0.05$, Dunnett test).

The results obtained with (\pm)MK-801 in the FST are shown in Fig. 2. A significant decrease in immobility was observed at the highest dose tested (0.1 mg/kg, SC).

These results can be compared with those obtained in the activity meter test (Fig. 3). A marked increase in locomotor activity was observed at the highest dose tested (0.3 mg/kg, PO). It can be noted that this dose was the same as the first dose that induced a significant decrease in immobility in the TST (Fig. 1).

Similar effects were observed with the (+) and (−) enantiomers of MK-801 on both tail suspension-induced immobility (Fig. 4) and locomotor activity (Fig. 5). Both enantiomers dose dependently reduced the duration of immobility and in-

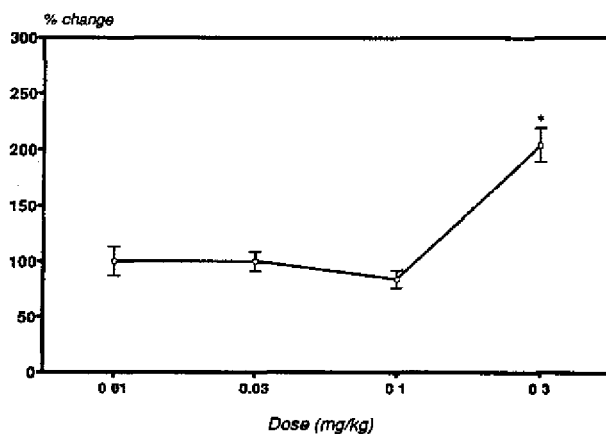


FIG. 3. Effects of (\pm)5-methyl-10,11-dihydroxy-5*H*-dibenzo(*a,d*)cyclohepten-5,10-imine (MK-801) (expressed as % change from control \pm SEM) on total locomotor activity during 30 min. The control mean was 141.3 ± 29.7 . (\pm)MK-801 was administered PO immediately before the 30-min testing period. *Significantly different from control group ($p < 0.05$, Dunnett test).

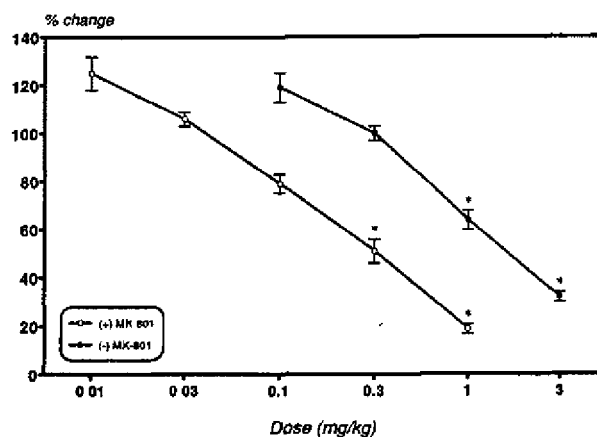


FIG. 4. Effects of (+)5-methyl-10,11-dihydroxy-5*H*-dibenzo(*a,d*)cyclohepten-5,10-imine (MK-801) and (−)MK-801 (expressed as % change from control \pm SEM) on immobility time in the tail suspension test (TST). The control means for (+)MK-801 and (−)MK-801 were 128.6 ± 17.2 and 125.2 ± 12.3 , respectively. The compounds were administered PO 30 min before testing. *Significantly different from control group ($p < 0.05$, Dunnett test).

creased locomotor activity. Both kinds of effect were observed in the same dose range. The (+) enantiomer was about five times more potent than the (−) enantiomer in both tests.

The effects of (\pm) MK-801 in the reserpine, apomorphine, and yohimbine tests are shown in Table 1. The compound had no significant effects on any parameter measured in a dose range similar to that found effective in the TST, FST, and locomotor activity tests (0.01–0.3 mg/kg, PO).

Results obtained with other NMDA receptor antagonists (AP7, kynurenic acid, and GDEE) in the TST are shown in

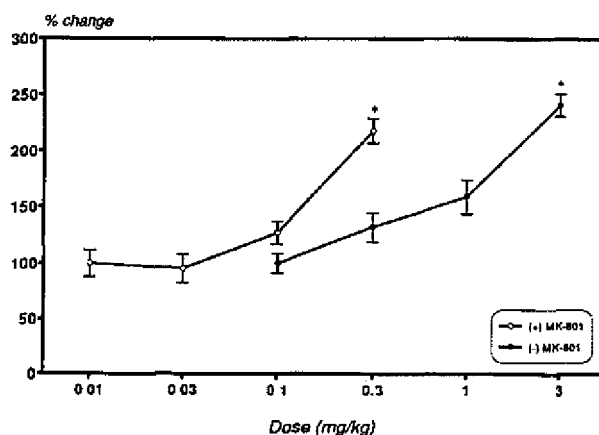


FIG. 5. Effects of (+)5-methyl-10,11-dihydroxy-5*H*-dibenzo(*a,d*)cyclohepten-5,10-imine (MK-801) and (−)MK-801 (expressed as % change from control \pm SEM) on total locomotor activity during a 30-min test. The control means for (+)MK-801 and (−)MK-801 were 139.3 ± 29.6 and 29.4 ± 29.4 , respectively. The compounds were administered PO immediately before the 30-min testing period. *Significantly different from control group ($p < 0.05$, Dunnett test).

TABLE 1
EFFECTS OF (±)MK-801 PO IN THE RESERPINE, APOMORPHINE, AND YOHIMBINE TESTS

Test	Drug	Dose	Parameter Measured			
			Mean Rectal Temperature (°C)			
			-4 h	0	1 h	2 h
Reserpine	Control	—	37.65 (±0.26)	32.1 (±0.36)	31.72 (±0.70)	31.43 (±0.85)
	(±)MK-801	0.01	37.67 (±0.27)	32.5 (±0.47)	30.98 (±0.96)	30.58 (±0.95)
		0.03	37.60 (±0.29)	32.2 (±0.36)	31.70 (±0.47)	31.43 (±0.66)
		0.1	37.75 (±0.23)	32.7 (±0.19)	30.92 (±0.85)	30.95 (±1.05)
		0.3	37.82 (±0.23)	32.1 (±0.47)	31.72 (±0.44)	31.48 (±0.47)
			Mean Rectal Temperature (°C)			
			- 30 min		+ 30 min	
Apomorphine	Control	—	36.95 (±0.24)		31.77 (±0.29)	
	(±)MK-801	0.01	37.12 (±0.26)		31.38 (±0.40)	
		0.03	36.73 (±0.21)		31.03 (±0.38)	
		0.1	36.93 (±0.09)		32.13 (±0.47)	
		0.3	36.72 (±0.25)		31.23 (±0.27)	
			Lethality (%)			
			+ 24 h			
Yohimbine	Control	—	10			
	(±)MK-801	0.01	40			
		0.03	0			
		0.1	0			
		0.3	0			

Rectal temperature (°C) was measured as described in the Method section. Lethality in the yohimbine test was calculated 24 h after drug administration.

Table 2. None of the compounds demonstrated any significant decrease in immobility time in the dose range tested.

DISCUSSION

The present studies demonstrate that the noncompetitive NMDA receptor antagonist MK-801, both the racemate and the (+) and (-) enantiomers, significantly reduced the duration of immobility in both the TST and FST in mice, two behavioral tests currently used for the detection of antidepressants (3,19). These findings are in accordance with those observed by Trullas and Skolnick (29) and similar to those obtained with classical antidepressants such as imipramine. On the other hand, our findings suggest that the reduction in immobility induced by MK-801 in the two tests is related to the marked increase of locomotor activity observed at the same doses. This stimulating effect of MK-801 has already been reported (11,14).

The psychomotor stimulant activity of MK-801 may well be related to its activity on brain DA metabolism (11). Although having no direct action on DA receptors, current data (14) suggest that MK-801 may facilitate DA transmission indirectly, perhaps via an open-channel block of glutamatergic neurotransmission. On the other hand, the locomotor stimulation observed in our experiments does not appear to be related to the PCP-like stereotypies (head weaving and circling behav-

iour) observed by ourselves and others (1,13,14) at higher doses. Indeed, the doses studied in the present experiments were chosen to clearly dissociate locomotor stimulation from these stereotyped phenomena.

Our findings indicate that the (+) isomer of MK-801 has qualitatively, but not quantitatively, similar effects to the (-) isomer. The difference between the enantiomers indicates that (+)MK-801 was about five times more potent than the (-) enantiomer. This phenomenon of receptor stereospecificity has also been reported with other functional antagonists at the NMDA receptor complex (AP7, AP5) in relation to anticonvulsant effects (16,26,27). The fact that the racemate showed the most potent activity in the TST probably indicates an additive synergism of the enantiomers.

In contrast to MK-801, none of the other NMDA receptor antagonists tested (AP7, kynurenic acid, or GDEE) induced a reduction in the duration of immobility in the TST. A possible explanation could be the lack of any stimulating effect of the cited compounds. This lack of increased locomotor activity has already been published for AP7 (29). Another explanation could be that these compounds only poorly cross the blood-brain barrier. On the other hand, the doses of AP7 (50-200 mg/kg, SC) were in the range of those found active (40-200 mg/kg, IP) by Trullas and Skolnick (29), and the other two compounds, which were tested up to 1,000 mg/kg PO, have shown anxiolytic or anticonvulsant activity after peripheral

TABLE 2
EFFECTS OF AP7, KYNURENIC ACID, AND GDEE ON
IMMOBILITY TIME IN THE TST

Drug	Dose	Immobility (seconds)	% Change From Control
Control	—	160.5 ± 4.6	
AP7	50	170.4 ± 7.3	+ 6.17
	100	170.0 ± 7.5	+ 5.92
	200	193.7 ± 11.5	+20.69
Control	—	149.1 ± 3.6	
Kynurenic acid	10	164.4 ± 4.8	+10.25
	100	101.2 ± 6.4	-32.13
	1,000	177.9 ± 3.8	+19.31
Control	—	136.1 ± 3.6	
GDEE	10	165.1 ± 4.8	+23.30
	100	117.1 ± 6.4	-13.96
	1,000	151.2 ± 3.8	+11.09

AP7 was administered SC and kynurenic acid and GDEE PO 30 min before testing. Immobility was measured as described in the Method section.

administration (12,31). These findings suggest therefore that the decrease in immobility by MK-801 and enantiomers in the TST is more related to the compound's intrinsic motor stimulant activity than to its activity at NMDA receptors.

This conclusion is corroborated by the absence of antidepressant-like activity of MK-801 in the reserpine, apomor-

phine, and yohimbine tests. Although these tests are based upon drug interactions and are therefore more closely predicted on particular mechanisms of drug action (mainly noradrenergic and serotonergic stimulation), they are generally sensitive to a large range of antidepressants (3,19). On the other hand, absence of activity in these tests alone could not be used as sole proof of ineffectiveness.

In contrast to the findings reported by Trullas and Skolnick (29), we found that AP7 was completely inactive in reducing the duration of immobility in the TST. This difference could be due to strain differences. Indeed, Trullas and coworkers (28) concluded that "performance of the TST as an animal model of depression is under specific genetic control." Other authors have commented on the importance of genetic aspects both for glutamate binding to NMDA receptors (17) and for the general pharmacological profile of NMDA antagonists (13). On the other hand, further studies in our laboratory (data not shown) obtained results similar to those presented here using NMRI and NIH Swiss strains.

In conclusion, the present results suggest that the apparent antidepressant effects of MK-801 in the TST and FST may not result from the compound's activity at the NMDA receptor complex but may be due to some DA-related motor stimulant activity of MK-801. A definitive answer as to whether functional antagonists at the NMDA receptor complex represent potential antidepressants cannot be given. The present data demonstrate both the weakness of traditional pharmacological interaction models for predicting the clinical effects of novel kinds of compound such as MK-801 and suggest the usefulness of conducting supplementary tests for detecting eventual false positives.

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Brief Report

A Double-Blind, Placebo-Controlled Study of Memantine in the Treatment of Major Depression

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► Abstract

OBJECTIVE: This study was designed to assess possible antidepressant effects of memantine, a selective *N*-methyl-D-aspartate (NMDA) receptor antagonist in humans. **METHOD:** In a double-blind, placebo-controlled study, 32 subjects with major depression were randomly assigned to receive memantine (5–20 mg/day) (N=16) or placebo (N=16) for 8 weeks. Primary efficacy was assessed by performance on the Montgomery-Åsberg Depression Rating Scale (MADRS). **RESULTS:** The linear mixed models for total MADRS scores showed no treatment effect. **CONCLUSIONS:** In an 8-week trial, the low-to-moderate-affinity NMDA antagonist memantine in doses of 5–20 mg/day was not effective in the treatment of major depressive disorder.

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► Introduction

Increasing evidence suggests that the glutamatergic system may be involved in the pathophysiology and treatment of depression. This includes the delayed, indirect effects of many antidepressants on the glutamatergic system, as well as the antidepressant effects of *N*-methyl-D-aspartate (NMDA) receptor antagonists in animal models of depression and glutamatergic modulators in humans (reviewed in reference 1). However, it remains unclear what aspects of glutamatergic modulation are necessary for antidepressant effects to occur (i.e., direct inhibition of the release of glutamate, direct effects at the ionotropic [NMDA, AMPA] or metabotropic receptors, or some combination of these mechanisms). In this context, it is noteworthy that a series of candidate glutamatergic drugs are available for human use; these agents allow for a more precise dissection of the role of the glutamatergic system in mood disorders (1).

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We first tested the glutamatergic modulator riluzole (an inhibitor of glutamate release) and found it to have antidepressant properties in patients with treatment-resistant major depression (2) and bipolar depression (3). In the present study, we sought to determine if selective antagonism of the NMDA receptor alone produces antidepressant effects. Berman et al. (4) found that a single dose of the high-affinity NMDA receptor antagonist ketamine resulted in a rapid—albeit transitory—antidepressant effect in patients with major depression. Unfortunately, ketamine's psychotomimetic effects preclude its use as a chronic antidepressant. Memantine is a low-to-moderate-affinity noncompetitive NMDA receptor antagonist (5) that is currently approved by the U.S. Food and Drug Administration for the treatment of Alzheimer's disease. In contrast to ketamine, memantine is devoid of psychotomimetic effects at therapeutic doses (5–20 mg/day) (5).

The objective of this study was to examine in a controlled study the efficacy and safety of the selective NMDA antagonist memantine in the treatment of major depression.

► Method

Men and women 18 to 80 years old who were outpatients with a diagnosis of major depressive disorder, recurrent, without psychotic features, diagnosed according to the Structured Clinical Interview for DSM-IV Axis I Disorders, were eligible to participate in the study. Subjects were required to have a Montgomery-Åsberg Depression Rating Scale (MADRS) score of ≥ 22 at screening and at the start of medication treatment (baseline).

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The patients included in the study had been free of comorbid substance abuse or dependence for 3 months, were free of acute medical illnesses, and were judged clinically not to be at serious risk for suicide. Comorbid axis I anxiety disorder diagnoses were permitted. The study was approved by the National Institute of Mental Health Institutional Review Board.

Written informed consent was obtained from all patients after the procedures had been fully explained.

Patients were evaluated on a weekly basis with the MADRS (primary efficacy measure), the Clinical Global Impression (CGI) severity scale, and the Hamilton Anxiety Rating Scale (HAM-A). After a 2-week single-blind placebo lead-in phase and drug-free period, participants were randomly assigned to double-blind treatment with memantine or placebo for 8 weeks. Subjects with a decrease of more than 20% in MADRS score during the 2-week drug-free period were excluded. Memantine was started at 5 mg/day and increased by 5 mg/week as tolerated up to a maximum of 20 mg/day. Zolpidem 5–10 mg/day was given as needed for insomnia (no more than three times per week) but not within 8 hours of ratings. No other psychotropic medication was allowed. Clinical response was defined as a decrease of 50% or greater in MADRS score from baseline. No structured psychotherapy was permitted during the trial. Treatment compliance was monitored by capsule counts.

Linear mixed models were used to evaluate changes in MADRS, CGI severity scale, and HAM-A scores. Fisher's exact test was used to evaluate categorical outcomes.

► Results

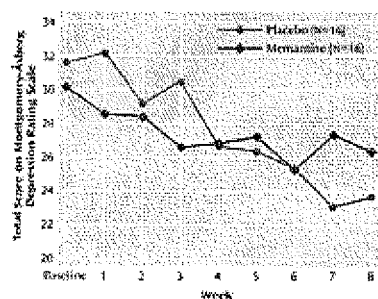
Thirty-two patients (16 men, 16 women; mean age=46.6 years, SD=10.8) were randomly assigned to receive memantine (N=16) or placebo (N=16). Eight subjects did not complete the placebo lead-in phase because of noncompliance with research procedures (N=4), significant worsening of depression (N=1), or an improvement of more than 20% in MADRS scores (N=3). One subject with early Alzheimer's disease who had a long history of major depressive disorder before the onset of the dementing process was included. Completion rates were 81% (N=13) for memantine and 81% (N=13) for placebo. Noncompletion in the memantine group was attributed to worsening at weeks 3 and 4 (N=2) and an adverse event at week 7 (N=1). Noncompletion in the placebo group was attributed to worsening at week 3 (N=1), an adverse event at week 3 (N=1), and withdrawal of consent at week 2 (N=1).

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There were no significant differences between the two groups in age (memantine: mean=47.1 years, SD=12.3; placebo: mean=46.1 years, SD=9.4), number of women in the group (memantine: N=9 [56%]; placebo: N=7 [44%]), current comorbid anxiety disorder (memantine: N=3 [19%]; placebo: N=2 [13%]), taking antidepressants at the time of screening (memantine: N=4 [25%]; placebo: N=3 [19%]), or previous failure to respond to an adequate antidepressant trial (memantine: N=4 [25%]; placebo: N=3 [19%]). Patients received memantine at a mean dose of 19.4 mg/day (SD=2.5) (15 [94%] patients achieved 20 mg/day) for a mean duration of 7.4 weeks (SD=1.5). Only four subjects took zolpidem as needed (two in the memantine group and two in the placebo group).

The linear mixed models for total MADRS, CGI severity scale, and HAM-A scores showed no treatment effect (MADRS: $F=0.01$, $df=1, 31$, $p=0.91$ [Figure 1]; CGI severity scale:

$F=0.05$, $df=1, 32$, $p=0.82$; HAM-A: $F=0.42$, $df=1, 33$, $p=0.52$) or interaction (MADRS: $F=0.22$, $df=8, 141$, $p=0.11$; CGI severity scale: $F=0.96$, $df=8, 140$, $p=0.47$; HAM-A: $F=0.84$, $df=8, 117$, $p=0.57$).



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Figure 1. Mean Change in Montgomery-Åsberg Depression Rating Scale Total Scores From Baseline in Patients With Major Depression Who Were Treated With Memantine or Placebo for 8 Weeks^a

^aResponse rates at week 8 for all patients were 13% (N=2) for memantine and 13% (N=2) for placebo.

► Discussion

To our knowledge, this is the first parallel-group, placebo-controlled trial of a selective NMDA antagonist in the treatment of major depressive disorder. This study failed to show that memantine, a low-to-moderate-affinity noncompetitive NMDA antagonist, has antidepressant effects in patients with major depression. Potential limitations of this study are the small number of subjects and low doses of memantine. The expected sample size would be 39 per group based on a moderate difference ($\phi=0.30$) in response rates. However, the observed effect was zero based on the response rates and favored placebo in the depression ratings. Although the present study group size of 32 has limited power to detect the significance of the expected effect, the effect size itself suggests no difference. A very large trial with similar response rates and depression levels would not show improvement on memantine. Given such an effect, the trial was ended early.

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Although it is possible that higher doses of memantine may have resulted in significant antidepressant effects, the dose chosen for the present study was judged to be sufficient to test the validity of the concept of NMDA receptor antagonism with memantine. The dose of memantine used in our study was based on 1) memantine's in vitro data of NMDA blockade, 2) NMDA's cognitive-enhancing effects in patients with Alzheimer's disease, and 3) similar memantine plasma levels mediating both neuroprotection in animal model studies and clinical effects in the treatment of Alzheimer's disease (5).

Despite these negative results, it remains possible that more potent NMDA blockade may

have utility in the treatment of depression for some patients. Memantine, in contrast to ketamine, has lower affinity for the NMDA receptor, has much faster open-channel blocking/unblocking kinetics, and exhibits a different type of channel closure (i.e., "partial trapping" as opposed to "trapping block" properties) (6). Such differences might explain the lack of antidepressant properties observed with memantine in the present trial.

► Footnotes

Received Nov. 2, 2004; revisions received March 2 and March 17, 2005; accepted April 18, 2005. From the Mood and Anxiety Disorders Program, National Institute of Mental Health, Department of Human Health Services, Bethesda, Md. Address correspondence to Dr. Zarate, 10 Center Dr., Mark O. Hatfield CRC, 7 SE, Rm. 7-3445, Bethesda, MD 20892; zaratec@mail.nih.gov (e-mail). Supported by the NIMH Intramural Research Program. Forest Laboratories supplied the study drug. None of the investigators in this study has a possible conflict of interest.

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